Metabolic and oxidative stress responses of the jellyfish *Cassiopea* sp.to changes in seawater temperature

Samir M. Aljbour[a,b,*], Martin Zimmer[a,b], Fuad A. Al-Horani[c,d], Andreas Kunzmann[a]

[a] *Leibniz-Zentrum für Marine Tropenforschung (ZMT) GmbH, Bremen, Germany*
[b] *Universität Bremen, Faculty of Biology and Chemistry, Bremen, Germany*
[c] *The University of Jordan, Aqaba, Jordan*
[d] *Marine Science Station, Jordan*

ABSTRACT

Jellyfish blooms might be driven by the alterations in seawater temperature (SWT) associated with climate change. The physiological responses of jellyfish to changing SWT, however, are poorly understood. Therefore, we asked the question: how do sudden changes (± 6 °C) in SWT affect the physiological performance of the jellyfish *Cassiopea* sp.? We measured the changes in mitochondrial cellular respiration (i.e., in term of the electron transport system (ETS) activity), superoxide dismutase (SOD) activity, and lipid peroxidation (LPO) to assess the jellyfish's physiological performance. In acute treatments (2 h), ETS increased only in response to cooling (to 20 °C) while SOD remained unchanged. In response to chronic treatment (2 weeks), ETS, SOD and LPO increased, while body mass decreased in response to cold (20 °C). In contrast, the heat-treated (32 °C) jellyfish did not increase their metabolic demands nor show signs of oxidative stress (OS). Moreover, they gained body mass. Because chlorophyll-a remained unchanged in all chronic-treated jellyfish, the cold-induced OS is more likely due to cellular respiration, not photosynthesis. Overall, *Cassiopea* sp. seems more sensitive to decreases in SWT then to increases. Therefore, *Cassiopea* sp. might benefit from the future projected rises in SWT, which could result in increased population abundance and an expansion in geographic distribution. Overall, these finding add new physiological evidences on jellyfish tolerance and might be used as a framework for further studies aiming at better understanding of jellyfish physiology.

1. Introduction

Jellyfish are generally well known to tolerate wide ranges of environmental conditions. Global warming, for instance, is thought to partially drive the poorly understood increased incidence of jellyfish blooms (Graham, 2001; Mills, 2001; Purcell et al., 2007; Gambill and Peck, 2014). In most coastal and estuarine habitats, the causes and effects of jellyfish bloom are unknown. Furthermore, the jellyfish's physiological tolerance and responses to changing climate are poorly understood.

The upside-down jellyfish (genus *Cassiopea*) is a widely distributed scyphozoan in shallow tropical and sub-tropical marine habitats (Gohar and Eisawy, 1960; Welsh et al., 2009; Stoner et al., 2011, 2014). While the jellyfish is considered invasive and exotic in some coastal habitats (e.g. such as the Hawaiian Islands and the Mediterranean Sea, respectively; Holland et al., 2004; Özbek and Öztürk, 2015), it is a key organism in many reef habitats (e.g. it is essential in nutrient cycling;

Jantzen et al., 2010; Niggl et al., 2010). Unlike other scyphozoans, *Cassiopea* spp. are epibenthic and able to perform photosynthesis. The jellyfish incorporates photosynthetic zooxanthellae (algal symbionts of the genus *Symbiodinium*) within its mesoglea during the non-embryonic stages of the life cycle (Hofmann and Kremer, 1981). In addition to their main roles in nutrient exchange; the zooxanthellae play essential roles in *Cassiopea*'s metamorphoses (i.e. strobilation of *Cassiopea* polyps; Rahat and Adar, 1980; Hofmann et al., 1996). Strobilation is a bottleneck asexual reproductive biology, which results in the production of free swimming ephyrae (small medusae) from sessile polyps.

Jellyfish are well known for high reproduction and feeding rates (i.e., under appropriate conditions) and can have important impacts on prey communities (Breitburg et al., 1997; Mills, 2001; Pitt et al., 2009). Furthermore, jellyfish blooms might have adverse effects on fisheries and fish stocks, marine habitats, tourism and many other industries that can cause substantial monetary losses each year (Tinta et al., 2012; Gordoa et al., 2013; Ghermandi et al., 2015; Qu et al., 2015). In

contrast to pelagic scyphozoans, only very few studies addressed *Cassiopea* spp. roles in jellyfish bloom. For example, Stoner et al. (2011, 2016) found that *Cassiopea* were larger and more abundant in human-impacted coastal habitats compared to protected habitats. While the authors have not provided any mechanistic explanations (i.e., in physiological terms) for these observations, Aljbour et al. (2017, 2018) could experimentally demonstrate some physiological clues at *Cassiopea*'s tolerance to anthropogenic disturbances and elevated temperature as well.

Seasonal rises in sea water temperature (SWT) affect marine organisms in many different ways. In *Cassiopea*, for example, rising SWT is a prerequisite for polyps' strobilation (Rahat and Adar, 1980; Hofmann et al., 1996). In the Yellow Sea, rises in SWT have been assumed as the main cause of jellyfish blooms at the Chinese side (Wei et al., 2015). In contrast to jellyfish, rises in SWT above the normal seasonal range could have detrimental effects on other marine invertebrates. In corals, for example, few degrees above the highest normal seasonal SWT could cause irreversible bleaching (Lesser et al., 1990; Donner et al., 2017), a result of temperature-mediated oxidative stress. Under stressful conditions (e.g. high SWT), marine organisms might suffer from oxidative stress (OS) due to elevated formation of reactive oxygen species (ROS; Regoli and Giuliani, 2014). OS, a state of imbalance between cellular anti-oxidants and ROS intracellular levels, could result in irreversible cellular damage. ROS are highly reactive, they attack all biological molecules (e.g. resulting in lipid peroxidation, DNA damage, and protein oxidation) and could cause cellular damage or death if not detoxified promptly (Lesser, 2006; Regoli and Giuliani, 2014). Whereas low levels of ROS play crucial roles in normal cellular metabolism and in fighting pathogens (Wilson et al., 2017), high levels could be lethal in some cases. In aerobically respiring organisms, ROS are normally-formed by-products associated with the mitochondrial electron transport system (ETS; Speakman and Selman, 2011). Superoxide (O_2^-) is the first formed and one of most reactive ROS. Intracellular concentrations are usually kept under control by superoxide dismutase (SOD) detoxifying activity (McCord and Fridovich, 1969). It is estimated that ca. 0.3–3% of O_2 respired in mitochondria is turned into O_2^- (Speakman and Selman, 2011). Therefore, while aerobic respiration is considered the main desirable production of energy used for cellular maintenance and growth (Relexans, 1996), it is the main source of ROS as well (Fridovich, 1975; Nohl et al., 2004; Speakman and Selman, 2011). Under high metabolic energy demand (e.g., under stress), ROS formation could exceed SOD detoxifying activity (Boveris and Chance, 1973; Speakman and Selman, 2011). Overall, antioxidant systems (e.g., SOD), ROS, and metabolic rate are all directly correlated and could determine the fate of an organism under stress (McCord and Fridovich, 1969; Fridovich, 1975; Lesser, 2006; Regoli and Giuliani, 2014).

Our understanding of jellyfish physiology and thermal tolerance is limited by lack of research on this topic. Therefore, how global warming might affect the jellyfish population is unclear. This is the first study on *Cassiopea* aiming at better understanding of its physiological performance in response to anomalies in SWT. Therefore, we asked the following questions: (1) how does *Cassiopea*'s metabolic demand respond to sudden changes in SWT? (2) Does *Cassiopea* experience OS upon exposure to sudden changes in SWT? (3) Does the jellyfish acclimate well during two weeks of incubation at the selected SWT? In order to answer these questions, we measured the following parameters in the jellyfish's tissues: (1) ETS and SOD enzyme activities; (2) tissue levels of malondialdehyde (MDA; commonly known as a marker of OS and the antioxidant status) and chlorophyll *a* (Chl a). The findings of this research bring new physiological evidences on *Cassiopea* tolerance/sensitivity to changing SWT and could provide a framework for understanding its fate under the possible future climate changes.

2. Materials and methods

2.1. Experimental organisms and experimental design

Forty-eight *Cassiopea* sp. medusae with a bell diameter of 3.5–5.2 cm and body mass of 3.4–7.1 g were used in this study. Medusae were raised for 3–4 months from ephyrae collected from a strobilating polyp culture kept for years at the MAREE facilities at the Leibniz-Zentrum für Marine Tropenforschung (ZMT), Bremen. The experimental organisms were selected as apparently morphologically healthy (i.e. no signs of pitched bells or lost arms, etc.) and of similar size. Prior to the experiment, medusae had been acclimated for two weeks in a single aquarium tank (ca. 120 l) with a recirculation system (salinity 40.2 ± 0.2, temperature 25.9 ± 0.1 °C; the typical salinity and temperature for *Cassiopea* sp. in the Gulf of Aqaba, Red Sea), 12:12 h light/dark cycle, and fed twice a week with freshly hatched *Artemia* nauplii.

Two sets of experiments were used to test *Cassiopea's* sp. responses to thermal treatments (cold/heat, 20 °C and 32 °C, respectively). Throughout all experiments, a control (salinity 40.2 ± 0.2, temperature 25.9 ± 0.1 °C) treatment was always run in parallel. The cold treatment (a drop by 6 °C from the control) aims to mimic the effect of a cold front in shallow coastal waters, such as could occur during upwelling, or as a result of translocation by aquaculture activities or ballast water. The heat treatments (an increase by 6 °C from the control) are oriented more towards what might happen in a tidal pool or under long-term global warming.

First, in the acute (hereafter referred as temperature shock) treatment, eight medusae were transferred immediately into pre-cooled or -heated individual 1.0-L glass jars kept in an 80 l water bath, set at the same treatment temperature for 2 h. Medusae ($N = 8$, for each treatment and a control, respectively) were sampled after two hours. Second, in the chronic treatment, eight medusae were transferred as in the acute treatment, but were kept at the same temperature for two weeks. In this experiment, the water quality of each individual glass jar was maintained by daily replacing ca. 90% of its water, using freshly prepared artificial seawater at the same temperature.

2.2. Bell diameter and body mass measurements

Bell diameter was measured at full relaxation of the bell. Body mass was measured as follows: after measuring the bell diameter, jellyfish were gently taken out of water by hand. Excess water on the jellyfish surface was removed by gently shaking the jellyfish and then touching any obvious water droplet by an absorbent tissue. Jellyfish were immediately immersed in a beaker containing a known mass of water and weighed to the nearest 0.1 g. The absorbent tissue did not touch the jellyfish, and the water in the weighing beaker had the same physical (SWT) and chemical (salinity and pH) properties as the water in the incubation jars. These precautions are important to avoid inducing any unwanted kind of stress in the jellyfish.

2.3. Tissue sampling and homogenization

Individual oral arms were cut from the distal tip to the base. The arm's base is the point where they arise from the ring-shaped tissue, where oral arms are normally fused. This approach ensured easier and reproducible cutting procedure, which avoided interference with other tissues associated with oral base. Oral arm samples were immediately put in pre-weighed plastic microtubes (3–5 oral arms per tube) and snap frozen at −80 °C. Tissue homogenization in preparation for the biochemical analysis was done as follows: the frozen oral arm masses were measured and semi-thawed on ice. The semi-thawed tissue was transferred to clean empty ice-cooled small glass tubes (ca. 3–5 mL volume) and then dispersed using IKA®-ULTRA-TURRAX dispersers with ice-precooled probes for < 30 s. The resulting crude homogenates

were aliquoted by pipetting into three pre-weighed, pre-cold plastic microtubes and immediately snap frozen at $-80\,°C$. In the next days after crude homogenate preparation for each experiment, the ETS and SOD, chlorophyll-a (Chla), protein and MDA content were measured as described in detail below. For both ETS and SOD activity assays the following common homogenization practice was used, keeping in mind the different homogenization buffers used for each enzyme. First, in the same frozen tubes containing the crude homogenates assigned for ETS (or SOD) we added ice-cold ETS (or SOD) homogenization buffer (chemicals constituents is described in appendix A) equal to 3 times the mass of the crude homogenate (e.g. 0.2 g tissue in 0.6 mL buffer); then ca. 500 mg glass beads mixture (0.4 and 1.0 mm diameter) were added to each tube. Then the tissues were further homogenized using FastPrep®-24 tissue homogenizer for two cycles (speed: 4.0 M/S, TN: 12×15, 15 s) followed by 3 min cooling in ice after each cycle to avoid excessive heating. Second, homogenates were then centrifuged at 5000g (or 12,000 rpm in the case of SOD) for 5 min at 4 °C, and then the supernatants were pipetted into new microtubes and assayed.

2.4. Enzymatic assays and protein content measurements

2.4.1. ETS activity

ETS activity was assayed using the common INT (Iodonitrotetrazolium) reduction assay (Packard, 1971; Owens and King, 1975). In this assay, the rate of INT reduction in the presence of the nonionic detergent Triton X-100 is used as a measure of the electron transport activity and as an index of oxygen consumption rate. Briefly, 503 μL of 0.1 M KP$_i$, pH 8.5, 250 μL of 8 mM INT, and 167 μL of 7.2 mM NADH were added sequentially to a disposable plastic cuvette, stirred gently and incubated at 22 °C for 3 min in a dry block thermostat (see appendix A for the chemicals descriptions). And then 80 μL of the sample supernatant were added to start the reaction, and the increase in absorbance over time was followed at 490 nm for 5 min at intervals of 10 s. The slope of the change in absorbance over the middle 3 min was used in calculating ETS activity after blank-correction. The results were calculated based on the corrected slopes and presented in mg O_2 h^{-1} g^1 WM (see eqs. 1 and 2).

$$ETS\ [U\ g^{-1}WM] = \frac{\Delta A/min}{\varepsilon \cdot d} \times \frac{V.\ cuvette\ (\mu L)}{V.\ assayed\ (\mu L)} \times \frac{V.\ homo\ buffer(\mu L)}{Sample\ mass(g)} \tag{1}$$

where, ΔA/min: is the change in absorbance over the measurment time (i.e., the slope). ϵ: is the molar absorptivity or the extinction coefficient of INT-Formazan (i.e., 15.9 mM^{-1} cm^{-1}). d: is the path length of the light through the cuvette (usually 1.0 cm unless mentioned). $V.homo$ $buffer$: is the volume of the buffered used in homogenization. $V.sample$: 80 μL in paper.

ETS activity may be expressed in term of oxygen consumption rate as follows Cammen et al. (1990):

$$ETS\ [\mu mol\ O_2\ min^{-1}\ g^{-1}WM] = \frac{1}{2}\ ETS\ [U\ g^{-1}WM] \tag{2}$$

2.4.2. SOD activity

SOD activity was assayed by the competitive inhibition assay based on McCord and Fridovich (1969); and further modifications by Beyer and Fridovich (1987) and Vandewalle and Petersen (1987). In this assay, cytochrome C (Cyt-c) reduction by O_2^- generated by the xanthine-xanthine oxidase coupled system is competitively inhibited by the cellular SOD activity. One unit of SOD causes a 50% inhibition in the rate of cyt-c reduction in this system. Briefly, 830 μL of SOD-AB solution, 100 μL of 0.1 mM Cyt-c, 10 μL of 5 mM xanthine, and 10 μL (ca. 1.8 mU) of xanthine oxidase (XO) were added sequentially to a disposable plastic cuvette, stirred gently and incubated at 22 °C in for 3 min in a dry block thermostat (see appendix A for the chemicals descriptions). The volumes and concentrations of Cyt-c and XO were

chosen so that the reduction of Cyt-c causes an increase in absorbance at 550 nm (ΔA_{550}) by 0.025 $\pm$ 0.005 per minute. A 50 μL sample of the supernatant was added to start the competitive inhibition reaction which was followed at 550 nm for 3 min at intervals of 10 s using PerkinElmer-Lambda 35 photometer. The SOD activity was presented in U g^{-1}WM.

2.4.3. MDA content

MDA content was determined using the common method known as thiobarbituricacid (TBA)-reactive substances (TBARS), based principally on the protocol of Uchiyama and Mihara (1978) with slight modifications. Briefly, the following reagents were added sequentially: 1% H_3PO_4, 0.6% TBA (i.e. freshly prepared in water) and phosphate buffer saline (PBS) pH = 7.3 to the crude homogenate in 3:1:1:0.6 ratio for H_3PO_4: TBA: PBS: tissue mass in mg, respectively. Immediately, the reaction mixture was vortexed and incubated at 90 °C for 45 min. The reaction was stopped by incubation in ice after the 45 min incubation, centrifuged twice at 10,000g for 5 min to get clearer supernatant. The supernatant absorbance spectrum (400–700 nm) was measured in triplicates using a TECAN-infinite M200 PRO photometer. We have calculated the MDA content using the third derivative approach and MDA standards prepared using the same reagents used in the assay. Results were presented in nmol g^{-1}WM.

2.4.4. Chla content

Chla content was determined as follows: 96% ethanol was added at a ratio of 15:1 to the crude homogenate, vortexed and immediately incubated in dark at 4 °C for 24 h. Supernatants were cleared by centrifugation at 5000g for 10 min after which the absorbance at 750 and 665 nm were read using a PerkinElmer-Lambda 35 photometer. Chla contents were calculated using the HELCOM COMBIN formula (HELCOM 2017).

2.5. Statistical analysis

Each group has been compared to its control group for differences in the same treatment using "Welsh two sample t-test", two tailed and 0.05 were set as the significance level. In all treatmens $N = 8$, unless mentioned elsewhere.

3. Results

3.1. Loss/gain of body mass and size and ETS activity

Changes in body mass and diameter were measured only for the chronically-treated *Cassiopea*. While cold-treated *Cassiopea* experienced significant losses in body mass ($t = -8.6$, $p < 0.001$) and bell dimeter (t = -8.6, p < 0.001), the heat-treated jellyfish gained body mass ($t = -2.5$, $p < 0.05$) with no significant changes in bell diameter (Fig. 1).

Cellular respiration (i.e. ETS activity) showed contrasting responses in both acute and chronic treatments. In response to the acute treatments, while cold-treated jellyfish showed high ETS activity (up to 34% increase compared to the control; $t = 4.58$, p < 0.001), no changes in ETS were detected in heat-treated jellyfish ($t = 1.19$, $p > 0.1$; Fig. 2). In response to the chronic treatments, cold-treated jellyfish sustained high ETS activity ($t = 3.00$, $p < 0.01$), while heat-treated jellyfish showed ca. 22% drop in ETS activity compared to the control ($t = 2.58$, p < 0.05; Fig. 2).

3.2. Superoxide dismutase activity and lipid peroxidation (MDA content)

SOD activity did not change in all acute-treated jellyfish (Fig. 3). MAD content was not measured in acute-treated jellyfish due to technical reasons. In response to the chronic treatments, while cold-treated jellyfish showed 18% increase in SOD activity ($t = 3.31$, p < 0.01) and
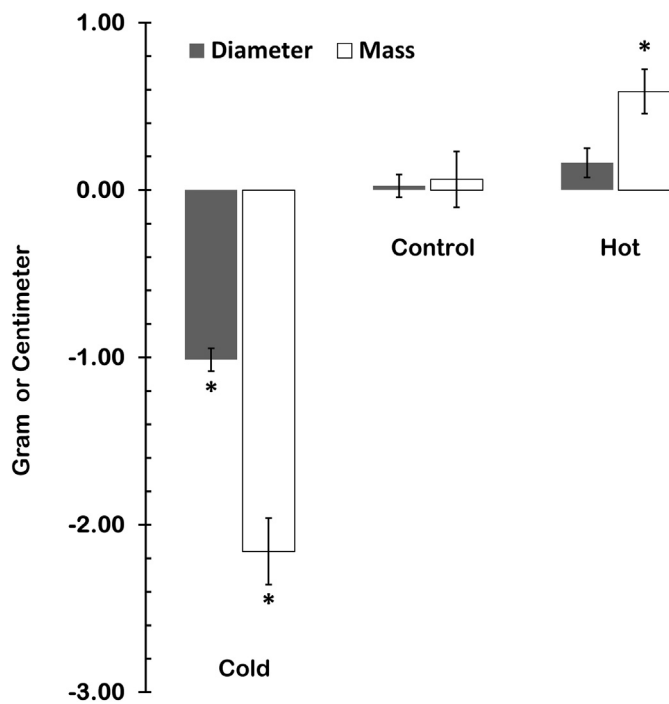
Fig. 1. Changes in bell diameter and mass of *Cassiopea* sp. in response to chronic cold/heat treatment. Bars represent the mean change in bell diameter or medusae mass $\pm$ SE, $n = 8$ in both control and cold treatments; $n = 7$ in hot treatment. Welch two sample *t*-test, and *p*-value < 0.05 is considered significant and is indicated by an asterisk above bars.
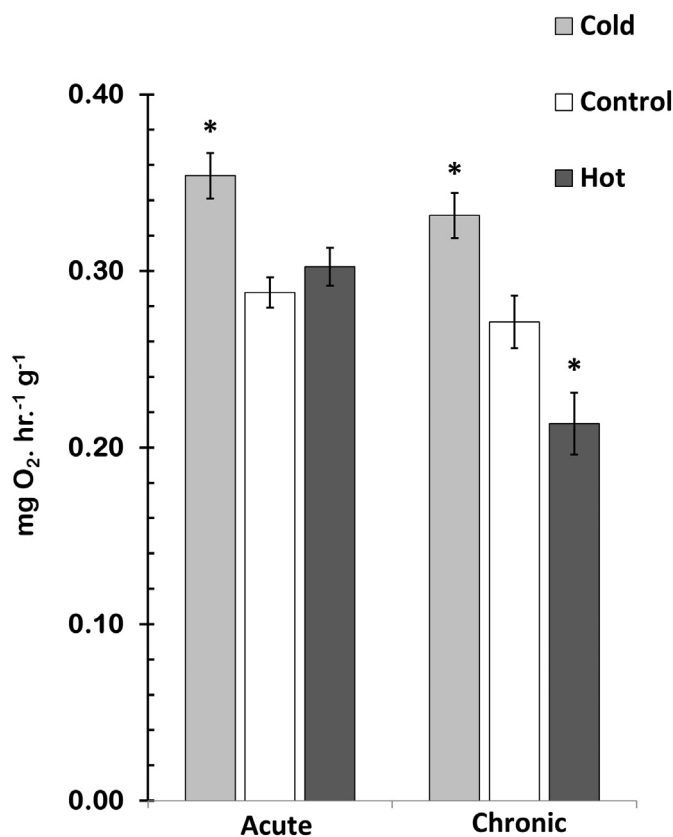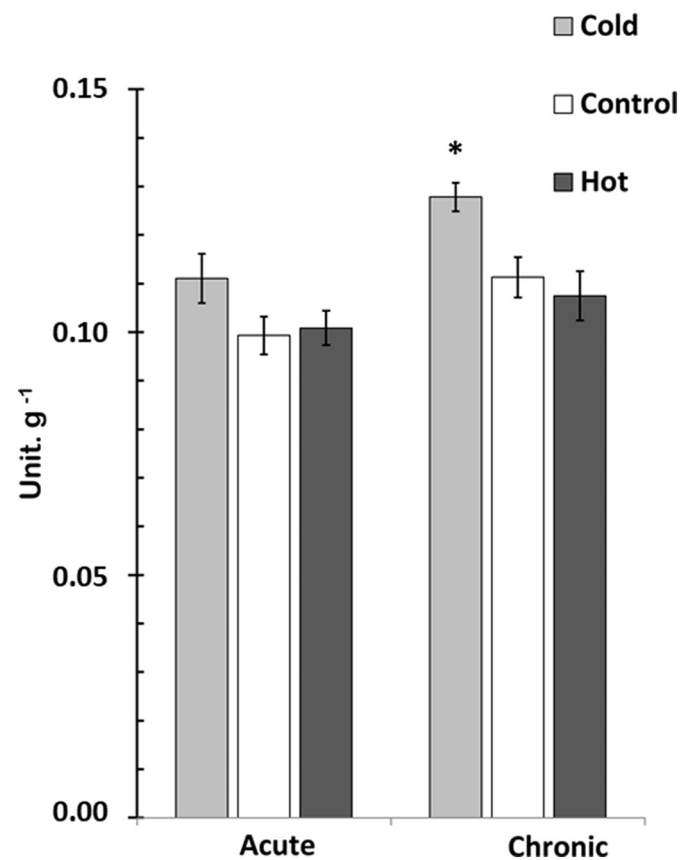

Fig. 3. Acute (left) and chronic (right) SOD responses to cold/heat treatments of *Cassiopea* sp. Bars represent the mean SOD activity per gram wet mass $\pm$ SE, $n = 8$. Two tailed Welch Two Sample t-test, and p-value < 0.05 is considered significant and is indicated by an asterisk above bars.

a 60% increase in MDA content ($t = 6.28$, $p < 0.0005$), both SOD activity and MDA content did not change in heat-treated jellyfish (Figs. 3 and 4).

3.3. Chla concentration response and its correlation to SOD activity

Chl*a* concentration (content) was measured only in the chronic-treated jellyfish. It did not change in all chronic treatments (Fig. 5). However, applying the Pearson's correlation test could reveal a positive correlation between SOD activity and Chla concentration in control (cor = 0.86, p < 0.01) and heat-treated jellyfish (cor = 0.77, p < 0.05).

4. Discussion

In the present study, changes in cellular respiration (ETS) and oxidative stress (OS) were used to demonstrate the physiological effects of sudden changes in SWT on *Cassiopea* sp. Experiencing OS and high ETS in the chronic cold-treated *Cassiopea*, but not in the heat-treated *Cassiopea*, might indicate that the jellyfish is more sensitive to decreases than increases in SWT. In contrast to the various responses to chronic treatments, the acute treatments induced only minor changes in ETS and OS. Therefore, the main conclusions drawn by this work are based on our findings in the chronic treatments.

Rapid fluctuations in SWT are well known for negatively affecting the performance of most marine organisms. For example, up to 60% increase in SOD activity was detected in zebrafish's (*Danio rerio*) brain tissues upon experiencing a sudden 10 °C decrease in SWT for 1 h (i.e. a cold shock), indicating a high intracellular ROS formation (Tseng et al., 2011). In contrast to zebrafish, the SOD activity in sea cucumber
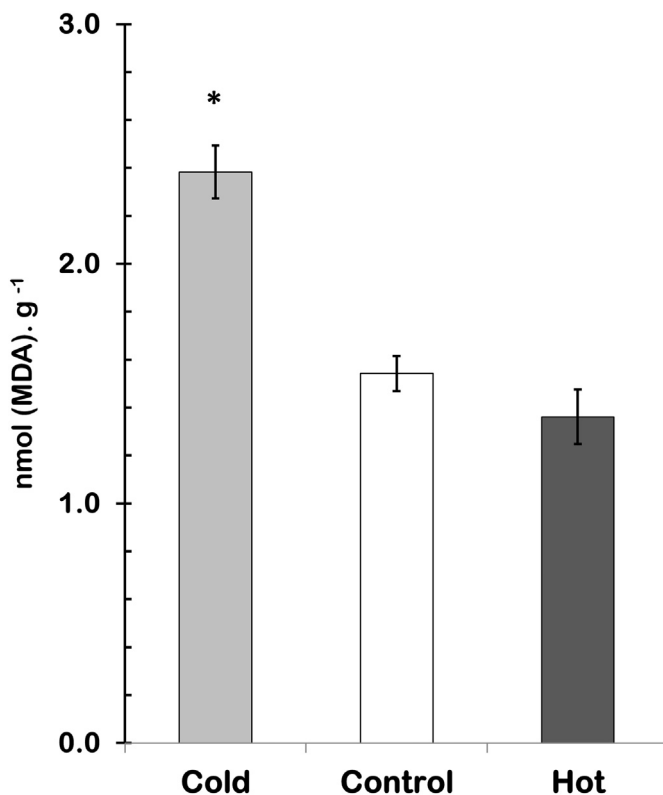

Fig. 2. Acute (left) and chronic (right) ETS responses to cold/heat treatments of *Cassiopea* sp. Bars represent the mean ETS activity per gram wet mass $\pm$ SE, $n = 8$. Two tailed Welch Two Sample t-test, and p-value < 0.05 is considered significant and is indicated by an asterisk above bars.

Fig. 4. Chronic lipid peroxidation (in terms of [MDA]) responses to cold/heat treatments of *Cassiopea* sp. Bars represent the mean [MDA] per gram wet mass $\pm$ SE, n = 8. Two tailed Welch Two Sample t-test, and p-value < 0.05 is considered significant and is indicated by an asterisk above bars.

Fig. 5. Chronic Chla responses to cold/heat treatments in *Cassiopea* sp. Bars represent the mean [Chla] per gram wet mass $\pm$ SE, $N = 8$. Two tailed Welch Two Sample t-test, and p-value < 0.05 is considered significant and is indicated by an asterisk above bars.

(*Apostichopus japonicus*) has significantly decreased in response to heat shock (i.e. at 32 °C for 1 h; Wang et al., 2008). Therefore, it is obvious that a rapid change in SWT could rapidly induce ROS formation and consequently increase the SOD activity in some marine organisms. In the current study, while the unchanged SOD activity in *Cassiopea* could not rule out the possibility of ROS formation in response to cold/heat-shocks, it might indicate that ROS did not build up to levels that area sufficient to induce a change in SOD activity. Overall, *Cassiopea* with an overall metabolic rate of ca. 0.2–0.3 mg O_2 h^{-1} g WM^{-1} (Fig. 2) might not show immediate reactions to a short-term treatment.

In the current study, while thermal shocks (i.e. short-term treatments) induced only minor responses in *Cassiopea*, chronic treatments (i.e. long-term treatments) induced major and contrasting responses in the jellyfish.

In living cells, polyunsaturated fatty acids (PUFA) of the membrane lipids are a major target of nucleophilic attack by ROS (which usually results in lipid peroxidation "LPO"; Ayala et al., 2014). Therefore, LPO is a commonly used biomarker of OS-mediated cellular damage (Ayala et al., 2014). In the polychaete worm *Diopatra neapolitana*, LPO was extensively used as an indicator of metal-induced OS-mediated cellular damage (Freitas et al., 2012). In the current study, high MDA levels (a proxy of LPO) and high SOD activity in the chronically cold-treated *Cassiopea*, reveal an elevated intracellular ROS levels. Furthermore, experiencing LPO activity in this treatment clarifies that the ROS levels have exceeded the SOD detoxifying potential.

In addition to suffering OS, the jellyfish suffered from a significant decrease (> 40%) in bell diameter and mass. While not part of our protocol, we have noticed that most jellyfish in this treatment have open bells (assumed to be an unhealthy sign) and showed reduced activity compared to the control. In the same treatment, high energy consumption was evident by the high ETS activity measured in these jellyfish. In the field, Fitt and Costley (1998) have noticed a decline in
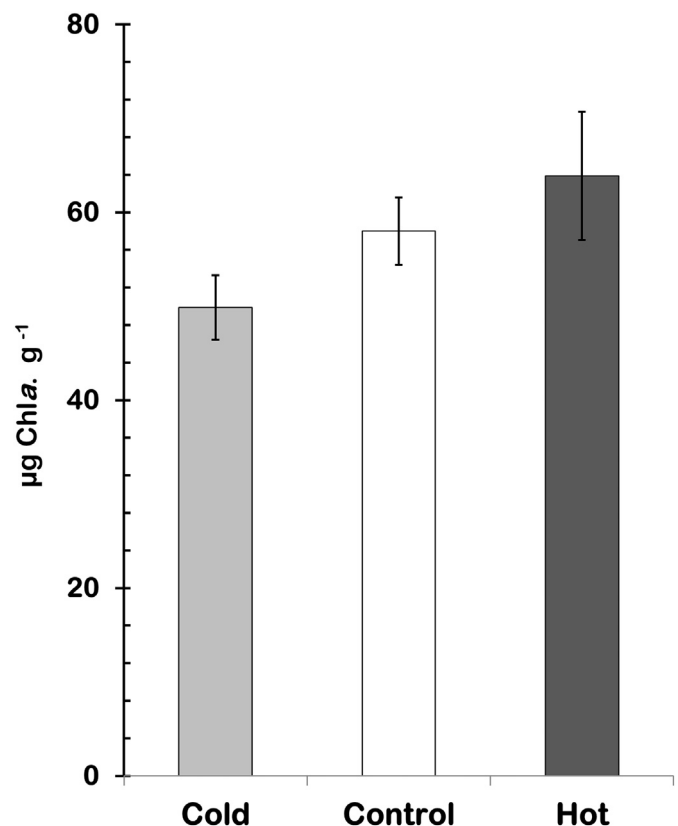
Cassiopea's population following the passage of a severe cold front through the shallow seagrass-mangrove flats in Grassy Key, Florida. Furthermore, many medusae had distended tentacles and asymmetric bells. The same authors have also found that a short-term cold (≤ 18 °C) treatment of the jellyfish's polyps resulted in decreased tentacle lengths and disturbed feeding abilities (Fitt and Costley, 1998). Overall, high energy consumption, experiencing OS and reduced body mass in the chronically cold-treated jellyfish clarify a reduced performance in response to cold temperature.

In general, SOD activity is positively correlated to photosynthetic rates, because photosynthesis is a major source of O_2^- (Fridovich, 1975). In *Cassiopea* and some other symbiotic cnidarians, SOD-Chla correlation has been already established (Dykens, 1984; Shick and Dykens, 1985). In the current study, SOD and Chla were positively correlated in control *Cassiopea*, but not in cold-treated jellyfish. Lack of correlation between SOD and Chla in the chronically cold-treated jellyfish could suggest that cellular respiration rather than photosynthesis is responsible for the leftover O_2^- in this treatment. This conclusion is further supported by the unchanged Chla and high ETS activity within the jellyfish's tissues of the cold treatment.

Jellyfish are generally seen as robust and noxious organisms; they are thought to benefit from global warming and eutrophication to bloom (Graham, 2001; Purcell et al., 2007; Gambill and Peck, 2014). For example, SWT rise (associated with global warming) and over-fishing has been suggested to be responsible for the increased populations of *Aurelia aurita* in the Seto Inland Sea of Japan (Uye and Ueta, 2004). Furthermore, Gambill et al. (2018) have shown that rising SWT could experimentally enhance the settlement of *Cyanea lamarckii* and *Chrysaora hysoscella* planulae. However, physiological studies on jellyfish responses to changing SWT and other anthropogenic disturbances are virtually overlooked. In general, rising SWT could impose negative

impacts (e.g., inducing OS, elevating energy expenditure, and causing death) on marine invertebrates (Lesser, 1997; Downs et al., 2002; Wang et al., 2008; Wolanski et al., 2017). For example, exposure of the sea cucumber *Apostichopus japonicas to* 32 °C for 16 h resulted in OS-mediated cellular damage and death (Wang et al., 2008). In this sea cucumber, OS was evident by the rapid change in catalase and SOD activities in the coelomic fluid. Unlike *A. japonicas and* most other invertebrates which usually experience OS at SWT slightly above 30 °C, *Cassiopea* did not show signs of being stressed, even at 32 °C. In the chronically heat-treated *Cassiopea*, unchanged SOD activity and MDA levels rule out the possibility of experiencing OS at this temperature. Furthermore, the jellyfish gained body mass and did not increase their energy expenditure. These findings overall suggest an enhanced performance of the jellyfish at 32 °C.

5. Conclusions

In light of our findings, we draw the following conclusions: 1- The jellyfish *Cassiopea* is more sensitive to cold (20 °C) than warm temperature (32 °C). 2- Oxidative stress and high energy consumption seem to limit *Cassiopea's* successful invasion into colder water bodies. 3- *Cassiopea* might benefit from the projected rises in SWT, where the jellyfish might increase its populations and geographical distribution. Since this is the first study on *Cassiopea* jellyfish to explore the physiological aspects of its tolerance/sensitivity to temperature changes, we encourage more research on this organism with longer experimental periods and different stress factors.

Acknowledgements

Appendix A. Brief descriptions on the preparation of some chemicals used in this paper

A.1. ETS chemicals

1- ETS assay buffer (ETS-AB): mix 0.1 M K_2HPO_4 with 0.1 M KH_2PO_4 to obtain potassium phosphate buffer (KPi) solution with pH 8.5 then keep it in the refrigerator, could be used as stock to prepare other reagents. 2- ETS homogenization buffer (ETS-HB); Dissolve the following chemicals: 0.45 g polyvinyl pyrolidone (PVP), 1.6 mL Triton X-10, and 2.7 mg $MgSO_4$ in 1.0 L final volume of 0.1 M KP_i, pH 8.5 (prepared according to Gopalan et al., 1996).

ETS substrates solutions: 1- Prepare 7.2 mM NADH in ETS-A containing 0.2% Triton X-100 (e.g., dissolve 25.5 mg NADH and 10.0 μL Triton-X100 in 5.0 mL KPi). Prepared fresh, stored on ice and exposure to direct intense light was avoided. 2- Prepare 8 mM INT (Iodonitrotetrazolium chloride) in 0.1 M KPi, pH 8.5 (e.g., dissolve 0.041 g INT in 10 mL KPi). Prepared fresh, stored on ice and the exposure to direct intense light was avoided.

A.2. SOD chemicals

1- SOD homogenization buffer (SOD-HB): mix 0.1 M K_2HPO_4 with 0.1 M KH_2PO_4 to obtain KPi solution with pH 7.4. 2- SOD assay buffer (SOD-AB): prepare 0.05 M KPi solution with 0.1 mM EDTA and adjust pH to 7.68 then keep it in the refrigerator.

SOD substrates solutions: 1- Prepare 0.1 mM Cyt-c in Milli-Q H_2O. 2- Prepare 5 mM xanthine solution by dissolving 3.04 mg xanthine in 4 mL of 0.1 M NaOH. 3- Prepare 1.8 mU of xanthine oxidase (XO) in SOD-AB.

A.3. Some reagents molecular masses (g.mol^{-1}) and source of purchase

NADH (709.41) from Merck, INT (505.7) from Sigma-Aldrich, xanthine (152.1) from Sigma-Aldrich. Cyt-c, XO, NaOH, EDTA, K_2HPO_4, KH_2PO_4, $MgSO_4$, PVP and all other chemicals mentioned in this paper were the product of Sigma-Aldrich.

References

Aljbour, S.M., Zimmer, M., Kunzmann, A., 2017. Cellular respiration, oxygen consumption, and trade–offs of the jellyfish *Cassiopea* sp. in response to temperature change. J. Sea Res. 128, 92–97.

Aljbour, S.M., Al-Horani, F.A., Kunzmann, A., 2018. Metabolic and oxidative stress responses of the jellyfish *Cassiopea* to pollution in the Gulf of Aqaba, Jordan. Mar. Pollut. Bull. 130, 271–278.

Ayala, A., Muñoz, M.F., Argüelles, S., 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4–hydroxy–2–nonenal. Oxidative Med. Cell. Longev. 2014 (ID 360438).

Beyer, W.F.Jr., Fridovich, I., 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal. Biochem. 161, 559–566.

Boveris, A., Chance, B., 1973. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. Biochem. J. 134 (3), 707–716.

Breitburg, D.L., Loher, T., Pacey, C.A., Gerstein, A., 1997. Varying effects of low dissolved oxygen on trophic interactions in an estuarine food web. Ecol. Monogr. 67 (4), 489–507.

Cammen, L.M., Corwin, S., Christensen, J.P., 1990. Electron transport system (ETS) activity as a measure of benthic macrofaunal metabolism. Mar. Ecol. Prog. Ser. 65, 171–182.

Donner, S.D., Rickbeil, G.J.M., Heron, S.F., 2017. A new, high–resolution global mass coral bleaching database. PLoS ONE 12 (4), e0175490.

Downs, C.A., Fauth, J.E., Halas, J.C., Dustan, P., Bemiss, J., Woodley, C.M., 2002. Oxidative stress and seasonal coral bleaching. Free Radic. Biol. Med. 33 (4), 533–543.

Dykens, J.A., 1984. Enzymic defenses against oxygen toxicity in marine cnidarians containing endosymbiotic algae. Mar. Biol. Lett. 5, 291–301.

Fitt, W.K., Costley, K., 1998. The role of temperature in survival of the polyp stage of the tropical rhizostome jellyfish *Cassiopea xamachana*. J. Exp. Mar. Biol. Ecol. 222, 79–91.

Freitas, R., Costa, E., Velez, C., Santos, J., Lima, A., Oliveira, C., et al., 2012. Looking for suitable biomarkers in benthic macroinvertebrates inhabiting coastal areas with low metal contamination: Comparison between the bivalve *Cerastoderma edule* and the Polychaete *Diopatra neapolitana*. Ecotoxicol. Environ. Saf. 75, 109–118.

Fridovich, I., 1975. Superoxide dismutase. Annu. Rev. Biochem. 44, 147–159.

Gambill, M., Peck, M.A., 2014. Respiration rates of the polyps of four jellyfish species: potential thermal triggers and limits. J. Exp. Mar. Biol. Ecol. 459, 17–22.

Gambill, M., McNaughton, S.L., Kreus, M., Peck, M.A., 2018. Temperature–dependent settlement of planula larvae of two scyphozoan jellyfish from the North Sea. Estuar. Coast. Shelf Sci. 201, 64–71. https://doi.org/10.1016/j.ecss.2016.08.042.

Ghermandi, A., Galil, B., Gowdy, J., Nunes, P.A.L.D., 2015. Jellyfish outbreak impacts on recreation in the Mediterranean Sea: welfare estimates from a socioeconomic pilot survey in Israel. Ecosyst. Serv. 11, 140–147.

Gohar, H.A.F., Eisawy, A.M., 1960. The biology of *Cassiopea andromeda* (from the Red Sea) (with a note on the species problem). Mar. Biol. Stn. Al–Ghardaqa. 11, 5–42.

Gopalan, G., Madon, S.P., Culver, D.A., Pappas, P.W., 1996. Measurement of metabolism in free juvenile fishes using electron transport system (ETS) enzyme assays. J. Sea Res. 128, 92–97.

Gordoa, A., Acuña, J.L., Farrés, R., Bacher, K., 2013. Burst feeding of *Pelagia noctiluca* ephyrae on Atlantic bluefin tuna (*Thunnus thynnus*) Eggs. PLoS ONE 8 (9), e7472.

Graham, W.M., 2001. Numerical increases and distributional shifts of *Chrysaora quinquecirrha* (Desor) and *Aurelia aurita* (Linnè) (Cnidaria: Scyphozoa) in the northern Gulf of Mexico. Hydrobiologia 451, 97–111.

HELCOM COMBIN; Manual for marine monitoring in the COMBINE Programme of HELCOM_PartC_AnnexC4, pp 261. Website:http://www.helcom.fi/action–areas/monitoring–and–assessment/manuals–and–guidelines/combine–manual, accessed on 20.05.2017.

Hofmann, K.D., Kremer, B.P., 1981. Carbon metabolism and strobilation in *Cassiopea andromeda* (Cnidaria: Scyphozoa) significance of endosymbiotic dinoflagellates. Mar. Biol. 65, 25–33.

Hofmann, D.K., Fitt, W.K., Fleck, J., 1996. Checkpoints in the life–cycle of *Cassiopea* spp.: control of metagenesis and metamorphosis in a tropical jellyfish. Int. J. Dev. Biol. 40, 331–338.

Holland, B.S., Dawson, M.N., Crow, G.L., Hofmann, K.D., 2004. Global phylogeography of *Cassiopea* (Scyphozoa: Rhizostomeae): molecular evidence for cryptic species and multiple invasions of the Hawaiian Islands. Mar. Biol. 145, 1119–1128.

Jantzen, C., Wild, C., Rasheed, M., El–Zibdah, M., Richter, C., 2010. Enhanced pore–water nutrient fluxes by the upside–down jellyfish *Cassiopea* sp. in a Red Sea coral reef. Mar. Ecol. Prog. Ser. 411, 117–125.

Lesser, M.P., 1997. Oxidative stress causes coral bleaching during exposure to elevated

temperatures. Coral Reefs 16, 187–192.

Lesser, M.P., 2006. Oxidative stress in marine environments: biochemistry and physiological ecology. Annu. Rev. Physiol. 68, 253–278.

Lesser, M.P., Stochaj, W.R., Tapley, D.W., Shick, J.M., 1990. Bleaching in coral reef anthozoans: effects of irradiance, ultraviolet radiation, and temperature on the activities of protective enzymes against active oxygen. Coral Reefs 8, 225–232.

McCord, J.M., Fridovich, I., 1969. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 244 (2), 6049–6065.

Mills, C.E., 2001. Jellyfish blooms: are populations increasing globally in response to changing ocean conditions? Hydrobiologia 451, 55–68.

Niggl, W., Naumann, M.S., Struck, U., Manasrah, R., Wild, C., 2010. Organic matter release by the benthic upside–down jellyfish *Cassiopea* sp. fuels pelagic food webs in coral reefs. J. Exp. Mar. Biol. Ecol. 384, 99–106.

Nohl, H., Gille, L., Staniek, K., 2004. The mystery of reactive oxygen species derived from cell respiration. Acta Biochim. Pol. 51 (1), 223–229.

Owens, T.G., King, F.D., 1975. The measurement of respiratory electron–transport–system activity in marine zooplankton. Mar. Biol. 30, 27–36.

Özbek, E.Ö., Öztürk, B., 2015. The new location record of *Cassiopea andromeda* (Forsskål, 1775) from Asin Bay, Gulf of Güllük, Muğla Aegean coast of Turkey. J. Black Sea/ Mediterr. Env. 21 (1), 96–101.

Packard, T.T., 1971. The measurement of respiratory electron transport activity in marine plankton. J. Mar. Res. 29, 235–244.

Pitt, K.A., Welsh, D.T., Condon, R.H., 2009. Influence of jellyfish blooms on carbon, nitrogen and phosphorus cycling and plankton production. Hydrobiologia 616, 133–149.

Purcell, J.E., Uye, S–.I., Lo, W–.T., 2007. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. Mar. Ecol. Prog. Ser. 350, 153–174.

Qu, C.–.F., Song, J.–.M., Li, N., Li, X.–.G., Yuan, H.–.M., Duan, L.–.Q., Ma, Q.–.X., 2015. Jellyfish (*Cyanea nozakii*) decomposition and its potential influence on marine environments studied via simulation experiments. Mar. Pollut. Bull. 97, 199–208.

Rahat, M., Adar, O., 1980. Effect of symbiotic zooxanthellae and temperature on budding and strobilation in *Cassiopeia andromeda* (Eschscholz). Biol. Bull. 159, 394–401.

Regoli, F., Giuliani, M.E., 2014. Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. Mar. Environ. Res. 93, 106–117. https://doi. org/10.1016/j.marenvres.2013.07.006.

Relexans, J.C., 1996. Measurement of the respiratory electron transport system (ETS) activity in marine sediments: state–of–the–art and interpretation. I. Methodology and review of literature data. Mar. Ecol. Prog. Ser. 136, 277–287.

Shick, J.M., Dykens, J.A., 1985. Oxygen detoxification in algal–invertebrate symbioses

from the Great Barrier Reef. Oecology 66, 33–41.

Speakman, J.R., Selman, C., 2011. The free–radical damage theory: accumulating evidence against a simple link of oxidative stress to ageing and lifespan. BioEssays 33, 255–259.

Stoner, E.W., Layman, C.A., Yeager, L.A., Hassett, H.M., 2011. Effects of anthropogenic disturbance on the abundance and size of epibenthic jellyfish *Cassiopea* spp. Mar. Pollut. Bull. 62, 1109–1114.

Stoner, E.W., Yeager, L.A., Sweatman, J.L., Sebilian, S.S., Layman, C.A., 2014. Modification of a seagrass community by benthic jellyfish blooms and nutrient enrichment. J. Exp. Mar. Biol. Ecol. 461, 185–192.

Stoner, E.W., Sebilian, S.S., Layman, C.A., 2016. Comparison of zooxanthellae densities from upside–down jellyfish, *Cassiopea xamachana*, across coastal habitats of the Bahamas. Rev. Biol. Mar. Oceanogr. 51 (1), 203–208.

Tinta, T., Kogovšek, T., Malej, A., Turk, V., 2012. Jellyfish modulate bacterial dynamic and community structure. PLoS ONE 7 (6), e39274.

Tseng, Y.–.C., Chen, R.–.D., Lucassen, M., Schmidt, M.M., Dringen, R., et al., 2011. Exploring uncoupling proteins and antioxidant mechanisms under acute cold exposure in brains of fish. PLoS ONE 6 (3), e18180. https://doi.org/10.1371/journal. pone.0018180.

Uchiyama, M., Mihara, M., 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem. 86, 71–278.

Uye, S.–.I., Ueta, U., 2004. Recent increase of jellyfish populations and their nuisance to fisheries in the inland Sea of Japan. Bull. Jpn. Fish. Oceanogr. 68 (1), 9–19.

Vandewalle, P.L., Petersen, N.O., 1987. Oxidation of reduced cytochrome c by hydrogen peroxide Implications for superoxide assays. FEBS Lett. 210 (2), 195–198.

Wang, F., Yang, H., Gao, F., Liu, G., 2008. Effects of acute temperature or salinity stress on the immune response in sea cucumber Apostichopus japonicas. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 151, 491–498.

Wei, H., Deng, L., Wang, Y., Zhao, L., Li, X., Zhang, F., 2015. Giant jellyfish *Nemopilema nomurai* gathering in the Yellow Sea– a numerical study. J. Mar. Syst. 144, 107–116.

Welsh, D.T., Dunn, R.J.K., Meziane, T., 2009. Oxygen and nutrient dynamics of the upside down jellyfish (*Cassiopea sp.*) and its influence on benthic nutrient exchanges and primary production. Hydrobiologia 635, 351–362.

Wilson, C., Muñoz–Palma, E., González–Billault, C., 2017. From birth to death: a role for reactive oxygen species in neuronal development. Semin. Cell Dev. Biol. https://doi. org/10.1016/j.semcdb.2017.09.012. In Press.

Wolanski, E., Andutta, F., Deleersnijder, E., Li, Y., Thomas, C.J., 2017. The Gulf of Carpentaria heated Torres Strait and the Northern Great Barrier Reef during the 2016 mass coral bleaching event. Estuar. Coast. Shelf Sci. 194, 172–181.